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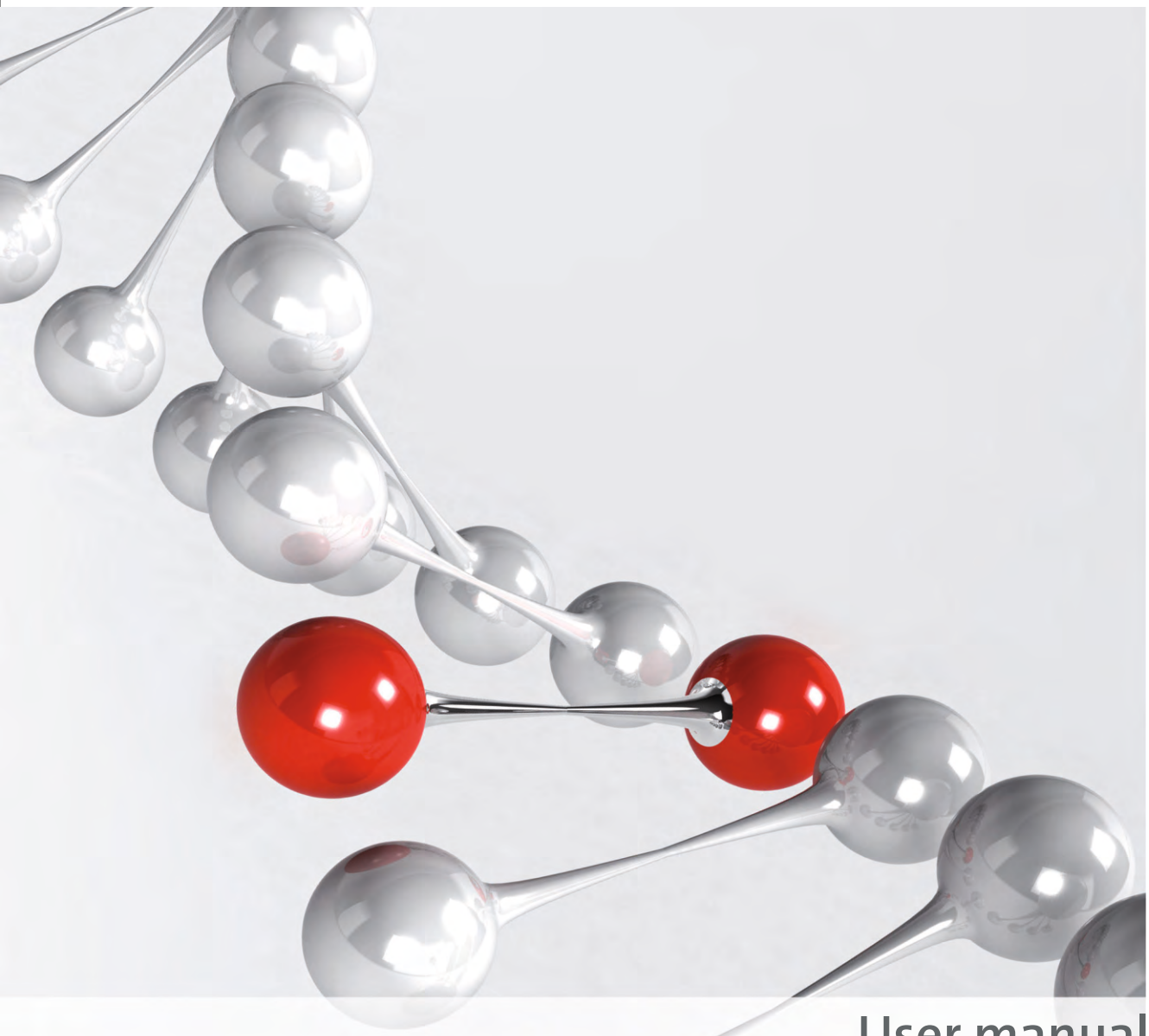

User manual
InviMag® Virus DNA/RNA Mini Kit
optimized for the InviMag® Rack

for purification of viral DNA and RNA from up to 200 µl serum, plasma, cell culture supernatant and other cell-free body fluids, biopsy samples and swab with magnetic beads

CE
IVD

REF 1440100X0

STRATEC Molecular GmbH, D-13125 Berlin

Instruction InviMag® Virus DNA/RNA Mini Kit

The **InviMag® Virus DNA/RNA Mini Kit** is the ideal tool using a combination of InviMag® and RTP® technologies on a magnetic rack for simultaneous isolation of high quality viral DNA and RNA from serum, plasma, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids, urine as well as from swabs or tissue biopsies for *in vitro* diagnostic use. Fresh or frozen plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*. The customer convenient RTP® technology simplifies the process handling, reduces the handling steps with infectious material and allows a process monitoring.

Furthermore viral DNA can be isolated from plant material.

The kit is neither validated for the isolation of genomic DNA nor for the isolation of total RNA.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Trademarks: InviMag®, RTP®, Invisorb®, Eppendorf®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, RTP® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Virus DNA/RNA Mini Kit

Store the **MAP Solution A** at 4 °C
Store dissolved **Proteinase K** at –20 °C.
Store all other kit components at room temperature (RT)!

	10 extractions	50 extractions	250 extractions
Catalogue No.	1440100900	1440100200	1440100300
Extraction Tubes	10	50	5 x 50
MAP Solution A	250 µl	1.1 ml	5.5 ml
Binding Solution (fill with 99.7% Isopropanol)	5 x 1 ml (ready to use)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Elution Buffer R	2 ml	15 ml	60 ml
Wash Buffer R1	15 ml (ready to use)	30 ml (final volume 60 ml)	125 ml (final volume 250 ml)
Wash Buffer R2	2 x 15 ml (ready to use)	2 x 12 ml (final volume 2 x 60 ml)	2 x 50 ml (final volume 2 x 250 ml)
Elution Tubes	20	2 x 50	10 x 50
Manual	1	1	1
Initial steps	**Wash Buffer R1** is ready to use **Wash Buffer R2** is ready to use **Binding Solution** is ready to use	Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 30 ml of 96 - 100 % ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 48 ml of 96 - 100 % ethanol to each bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!	Fill 120 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 125 ml of 96 - 100 % ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 200 ml of 96 - 100 % ethanol to each bottle **Wash Buffer R2** , mix thoroughly and always keep the bottle firmly closed!

Symbols

Lot number

Catalogue number

Date of manufacture

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit components, except the dissolved **Proteinase K** and the **MAP Solution A,** should be stored well sealed and dry at room temperature (RT) and are stable for at least 12 months under these conditions. **MAP Solution A** is stable for at least 6 months**.**

MAP Solution A: should be stored at 4 °C.

Proteinase K: Dissolved Proteinase K must be stored at –20 °C. Dividing Proteinase K into aliquots and storage at –20 °C is recommended.

Wash Buffer charged with ethanol should be stored at room temperature and should be appropriately sealed. If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature (up to 30°C).
Room temperature is defined as range from 15 – 30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Virus DNA/RNA Mini Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Virus DNA/RNA Mini Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviMag® Virus DNA/RNA Mini Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Virus DNA/RNA Mini Kit** is designed for a reliable and fast simultaneous isolation of high quality viral DNA and RNA from a wide range of clinical samples (from fresh or frozen human or mammalian serum, plasma, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids as well as from swabs or tissue biopsies), using the **RTP® technology**, magnetic beads, and a magnetic tool.

For reproducible high yields an appropriate sample storage and quick operation under the rules for RNA and DNA operation is essential. The purified viral DNA and/or RNA are ready to use for *in vitro* diagnostic analysis.

The isolation protocols as well as all buffers are optimized to provide high yield and purity of the extracted viral nucleic acids. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.
To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from stool sample, dried blood stains, nor from bacteria, fungi, parasites or the purification of total RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.
The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Virus DNA/RNA Mini Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Virus DNA/RNA Mini Kit** to which they apply, are listed below as follows:

Wash Buffer R1 contains guanidinthiocyanate which is an irritant.

Wash Buffer R1

danger
H302-312-332-412 EUH032 P273

Proteinase K (included in the Extraction Tube)

danger
H315-319-334-335 P280-305-351-338-310-405

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
in USA : 1 – 800 – 535 – 5053

Product characteristic of the InviMag® Virus DNA/RNA Mini Kit

Starting material	Yield	Time
up to 200 µl cell free body fluids; swab material, up to 400 µl rinse liquid from swab cell culture supernatants 1 x 10^6 mammalian cells; max. 10 mg tissue sample	depends on the sample storage and source sensitive recovery realizing amplification with sensitive detection assays from starting material including minimum 100 copies per ml ***Note:*** *The added Carrier RNA will account for most of the eluted RNA. Quantitative RT-PCR is recommended for determination of the viral RNA or DNA yield.*	about 20 - 25 min after lysis

The **InviMag® Virus DNA/RNA Mini Kit** is designed for a simultaneous rapid and economical preparation of viral DNA and RNA from a wide range of clinical samples, using a combination of **RTP® technology**, magnetic beads, and a magnetic tool.

The amount of purified viral DNA and RNA in the **InviMag® Virus DNA/RNA Mini Kit** depend on the sample type, the virus titer, sample source, transport, storage, and age.

The isolation process is based on a patented technology, the **InviMag® technology**, for isolation of viral RNA and DNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components. The kit uses further the patented RTP® technology, whose special feature is an **Extraction Tube** containing preformulated solid lysis reagent, **Proteinase K**, Carrier Nucleic Acids and a precisely calibrated amount of an internal **DNA Extraction Control** or on request an internal **RNA and DNA Extraction Control.** The technology allows the reduction of reagent preparation steps and of handling steps with infectious material.

Using the **Extraction Tube,** all types of samples are transferred into the Extraction Tube together with ddH_2O to adjust a final sample volume of 400 µl. The prefilled buffer and enzymes lyse the samples, stabilize the viral nucleic acids. After the lysis under higher temperature and shaking, binding buffer and magnetic beads are transferred to the lysate to enhance the selective viral RNA and DNA adsorption to magnetic particles, followed by washing steps and the final elution. The procedure requires minimized interaction by the user, allowing safe handling of potentially infectious samples. The procedures are designed to avoid sample-to-sample cross-contamination.

The advantage of the kit results from the simultaneous isolation of nucleic acids from DNA and RNA viruses. That enables the user to test the sample for all kinds of RNA and DNA viruses which are of interest after one preparation.

Yield and quality of isolated viral DNA and RNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer's specifications. Due to the high purity, the isolated viral DNA/RNA is ready to use for a broad panel of downstream applications or can be stored at –80°C for subsequent use.

- RT-PCR*
- PCR*
- Real-time PCR for quantitative and qualitative virus diagnostic

No toxic or hazardous chemicals like chaotropic components are used.

For the isolation of viral nucleic acids with spin filters from 200 µl starting material STRATEC Molecular offers the **RTP® DNA/RNA Virus Mini Kit** and the **Invisorb® Spin Virus RNA Mini Kit**.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

For the isolation of viral nucleic acids using magnetic particle in high-throughput format, STRATEC Molecular offers the **InviMag® Virus Kits/ KFmL** and the **InviMag® Virus Kits/ KF96** for use on a KingFisher mL and 96 robot. For vacuum or centrifuge based isolation of viral NA in 96 well format on different robotic stations STRATEC Molecular offers the **Invisorb® DNA Virus HTS 96 Kit** and the **Invisorb® RNA Virus HTS 96 Kit** (see ordering information, page 19).

For further information please contact: Phone: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries phone: +49 (0) 30 9489 2907 or your local distributor

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore it is essential, that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80°C. Viral RNA contained in such deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved Lysis Buffer in the Extraction TubeI or II for 1 h at room temperature, overnight at 4°C, and for long term storage at –80°C. Storage under deep frozen conditions is recommended.

Serum, plasma, urine, cerebrospinal fluid or other cell free body fluids, as well as cell culture supernatants, swabs, and stool samples can be stored on ice for 1 - 2 hours, for short time (up to 24 h) samples may be stored at -20°C. For long term storage, we recommend freezing samples at –80°C. Multiple thawing and freezing before isolating the viral RNA should be avoided.

Serum and plasma (and other cell-free body fluids)
Following centrifugation, plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*, can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C to –80°C in aliquots is recommended. Repeated freezing and thawing cycles must be avoided because denaturation and precipitation of proteins result in a decrease of the virus titer and thereby reduce the yield of the extracted viral RNA. Occurring cryoprecipitates can be pelleted by briefly centrifuging (6.800 x g for 3 min). The cleared supernatant should be removed, without disturbing the pellet, and processed immediately. This step will not reduce viral titers.

Tissue samples (biopsy material or frozen section)
Best results are obtained with fresh material or material that has been immediately flash frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced RNA yield. Use of poor quality starting material influences the RNA yield, too. The amount of purified RNA in the **InviMag® Virus DNA/RNA Mini Kit** procedure using up to 20 mg tissue sample, depends on kind of starting material. The thawing process could be proceed, e.g. directly in the **Extraction Tubes.**

Cell culture supernatants
Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C after winning of the cell culture supernatant. Repeated freezing and thawing of stored samples should be avoided can influence the sensitivity.

Swabs
The protocol works with fresh prepared swabs or rinsed liquid from swabs or mouth brushes. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C

Principle and procedure

The **InviMag® Virus DNA/RNA Mini Kit** procedure comprises following steps:

- Lysis of the virus particles in the prefilled Extraction Tube
- Binding the viral NA to the magnetic beads
- Washing of the magnetic beads and elimination of ethanol
- Elution of the viral nucleic acids

After lysis the viral NA bind to the magnetic beads, contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA/RNA is eluted in Elution Buffer R or water.
This manual contains 4 protocols.

Lysis
Samples are lysed under denaturing conditions at elevated temperatures.

Binding of the viral nucleic acids
After adding **Binding Solution** and **MAP Solution A** to the lysate in the **Extraction Tube**, the nucleic acids are bound to the surface of the magnetic beads.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer R1** and **R2**, while the nucleic acids remain bound to the magnetic beads

Elution
The nucleic acids are eluted from the beads using 120 µl **Elution Buffer R**.
The eluted nucleic acids are ready for use in different subsequent tests.

Yield and quality of viral DNA/ RNA
Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain viral RNA/ DNA and Carrier RNA, and amounts of Carrier RNA will greatly exceed amounts of viral nucleic acids.

Yields of viral RNA/ DNA isolated from biological samples are normally less than 1 µg and therefore difficult to determine photometrically.* Keep in mind that the Carrier RNA (5 µg per 200 µl sample) will account for most of the RNA present.
The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

** In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.*

Internal control (IC) / Extraction control

Internal Controls (IC) from the PCR assay provider can be used as extraction controls if the fragments are longer than 100 bp. In this case they have to be added after finalization of the lysis step.

Attention: don´t add directly these Internal Controls to the sample!

Important notes

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kit, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Important indications

Preparing viral RNA
When preparing viral RNA, work quickly during the manual steps of the procedure.
The Lysis Buffer in the Extraction Tube simplifies viral RNA isolation by combining efficient lysis of the starting material and the inactivation of exogenous and endogenous RNases. Extreme care should be taken to avoid contaminations with RNases when handling Elution Buffer R.

Storing samples
Frozen serum or plasma samples must not be thawed more than once. Repeated “freeze – thawing” leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids.

Carrier RNA
Carrier RNA serves two purposes. Firstly, it enhances the binding of viral acids to the MAP Solution A, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of Carrier RNA reduces the chance of viral nucleic acid degradation in the rare event that RNase or DNase molecules are not denaturated by the salts and detergents in the Lysis Buffer in the Extraction Tube.

Internal Controls
The use of an internal control is recommended when using the **InviMag® Virus DNA/RNA Mini Kit** in combination with diagnostic amplification systems. STRATEC Molecular provides the needed Control DNA or RNA Detection Assays.

Eluting viral RNA and DNA
For downstream applications, that require small starting volumes, elution volume may be reduced to 40 µl Elution Buffer R.

The volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the RTA Spin Filter. The volume of eluate recovered depends on the nature of the sample.

Preparing buffers and reagents

10 viral DNA/RNA-extractions:
Binding Solution, Wash Buffer R1 and **R2** are ready to use!
50 viral DNA/RNA-extractions:
Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 30 ml 96-100% ethanol to the bottle **Wash Buffer R1**. Add 48 ml 96-100% ethanol to each bottle **Wash Buffer R2**.
250 viral DNA/RNA-extractions:
Fill 120 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 125 ml 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml 96-100% ethanol to each bottle **Wash Buffer R2**.

Special instruction for samples with high virus titer

When using high titer samples please remember that depending on virus the system has an upper capacity limit. It may be necessary to dilute the sample in advance of the preparation. Samples with titer higher than 10^6 copies/ µl may be at this limit, but please remember that this depends on virus strain.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- ddH_2O
- Vortexer
- 96 – 100 % Ethanol
- Isopropanol *

*The **InviMag® Virus DNA/ RNA Mini Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from Carl Roth

*** Possible suppliers for Isopropanol:**

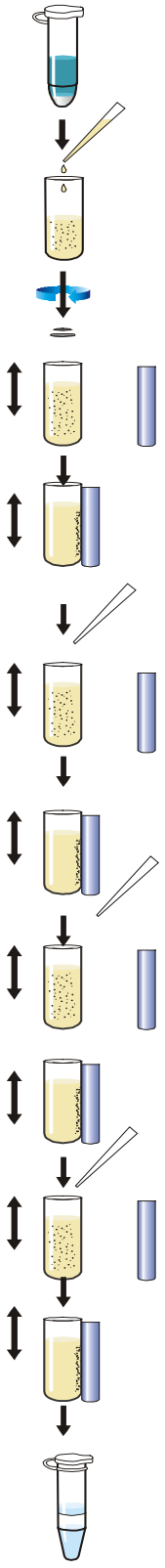
Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma
2-Propanol
Order no. 59304-1L-F

Scheme of the InviMag® Virus DNA/RNA Mini Kit

Please work quickly and perform all extraction steps at room temperature (RT)!



Please read protocols prior the start of the preparation carefully

transfer 200 µl sample into the provided Extraction Tubes
add 200 µl **dd H_2O**
for samples which have a smaller volume than 200 µl please fill up to a total volume of 400 µl with ddH_2O
incubate for 15 minutes at 65°C in a thermomixer
incubate for 10 minutes at 95°C in a thermomixer

for realization of the optimal binding conditions add 400 µl **Binding Solution** and 20 µl MAP Solution A
mix the sample completely
by pipetting up and down

binding of viral NA to magnetic particle

magnetic separation of beads
removal of lysed material

add 800 µl **Wash Buffer R1**
washing of the particle fixed viral NA

magnetic separation of beads
removal of the wash buffer eluate

add 800 µl **Wash Buffer R2**
washing of the particle fixed viral NA

magnetic separation of beads
removal of the wash buffer eluate
repeat this step

add 120 µl **Elution Buffer R**

magnetic separation of beads
transfer of the eluate in a new 1,5 ml Receiver Tube

pure viral RNA or DNA

Protocol 1: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from cell-free body fluids (serum, plasma, CSF, synovia, urine) and from cell culture supernatant (cell culture media)

Please read the instructions carefully and conduct the prepared procedure.

See also: Special instruction for samples with high virus titer page 12

1. Transfer 200 µl of the sample (serum, plasma, CSF, synovia, urine, cell culture) into the **Extraction Tube** and add 200 µl of ddH_2O. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C.
3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 minutes at 95°C. (These step leads to higher sensitivity for some viral strains.)
4. Transfer the **Extraction Tube** in the **Magnet Rack** without magnet and add 400 µl **Binding Solution** and 20 µl **MAP Solution A** and mix the sample by pipetting up and down for 4- 5 times

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Incubate for 5 min the mixture at RT. Then insert the **Magnetic Stripe** and incubate for further 5 min at RT. Remove supernatant by aspiration or by pipetting, then remove the **Magnetic Stripe**.
6. Add 800 µl **Wash Buffer R1** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, then remove the **Magnetic Stripe**.
7. Add 800 µl **Wash Buffer R2** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, then remove the **Magnetic Stripe**.

Repeat once this Step.

8. Insert the **Magnetic Stripe** and open the lid of the tube for 10 min to evaporate the ethanol completely.
9. Remove the **Magnetic Stripe**, then add 120 µl of the **Elution Buffer R**, mix by pipetting up and down for three times. Incubate at room temperature for 5 min.
10. Insert the **Magnetic Stripe** and incubate for further 3 min. Transfer the eluate to the 1.5 ml **Elution Tube**, centrifuge the tube for 3 min at 10.000 x g and transfer the supernatant to a new 1.5 ml **Elution Tube.** If no centrifuge is available, please put the vial again in the **Magnet Rack,** insert the **Magnetic Stripe** and incubate for further 5 min at RT. Transfer the supernatant in a new 1.5 ml **Elution Tube.** It contains the viral nucleic acids ready to use.

Important Notes:

1. *The viral nucleic acids can also be eluted with a lower volume of Elution Buffer R (depends on the expected amounts of viral nucleic acids). But pay attention that minimum volume for the elution is* ***30 µl and that this volume can reduce the maximum yield****. If quite large amount of viral nucleic acids are expected, the volume of Elution Buffer can be increased up to 200 µl.*

2. *After finishing the extraction protocol, the Elution Tube contains the extracted viral RNA/DNA. We recommend storage of the DNA at –20°C or –80°C especially for RNA.*

Protocol 2: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from swabs or 200 µl rinse liquid

Please read the instructions carefully and conduct the prepared procedure

See also: Special instruction for samples with high virus titer page 12

1a. Place the swab into the **Extraction Tube** and add 400 µl of ddH_2O. Close the cap and mix by vortexing for 10 s**.**

1b. Transfer 400 µl of rinse liquid, or of the stabilization media into the **Extraction Tube.** The stabilization medium shouldn't contain alcohol. Close the cap, mix by vortexing for 10 s**.**

2. Place the **Extraction Tube** into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C.

3. Place the **Extraction Tube** into another Thermomixer and incubate under continuously shaking for 10 minutes at 95°C. (These step leads to higher sensitivity for some viral strains.)

Important Note: *To get maximum yield of viral nucleic acids it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the Extraction Tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the Extraction Tube ahead of time will be lead to a dramatically reduced final yield! After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.*

4. Remove the swab and transfer the **Extraction Tube** with the lysate in the **Magnet Rack** without magnet. Add 400 µl **Binding Solution** and 20 µl **MAP Solution A** and mix the sample by pipetting up and down for 4- 5 times

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Positioning of the **Extraction Tube** in the **Magnet Rack** without magnet and incubate for 5 min the mixture at RT. Then insert the **Magnetic Stripe** and incubate for further 5 min at RT. Remove supernatant by aspiration or by pipetting, then remove the **Magnetic Stripe**.

6. Add 800 µl **Wash Buffer R1** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, and then remove the **Magnetic Stripe**.

7. Add 800 µl **Wash Buffer R2** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, and then remove the **Magnetic Stripe**.

Repeat once this Step.

8. Insert the **Magnetic Stripe** and open the lid of the tube for 10 min to evaporate the ethanol completely.

9. Remove the **Magnetic Stripe**, then add 120 µl of the **Elution Buffer R**, mix by pipetting up and down for three times. Incubate at room temperature for 5 min.

10. Insert the **Magnetic Stripe** and incubate for further 3 min. Transfer the eluate to the 1.5 ml **Elution Tube**, centrifuge the tube for 3 min at 10.000 x g and transfer the supernatant to a new 1.5 ml **Elution Tube.** If no centrifuge is available, please put the vial again in the **Magnet Rack**, insert the **Magnetic Stripe** and incubate for further 5 min at RT. Transfer the supernatant in a new 1.5 ml **Elution Tube.** It contains the viral nucleic acids ready to use.

Important Notes:

*1. The viral nucleic acids can also be eluted with a lower volume of Elution Buffer R (depends on the expected amounts of viral nucleic acids). But pay attention that minimum volume for the elution is **30 µl and that this volume can reduce the maximum yield**. If quite large amount of viral nucleic acids are expected, the volume of Elution Buffer can be increased up to 200 µl.*

2. After finishing the extraction protocol, the Elution Tube contains the extracted viral RNA/DNA. We recommend storage of the DNA at –20°C or –80°C especially for RNA.

Protocol 3: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from tissue biopsy

Please read the instructions carefully and conduct the prepared procedure

See also : Special instruction for samples with high virus titer page 12

1. Transfer 1mg up to – max. 10 mg of the tissue biopsy into the **Extraction Tube.** Add 400 µl of ddH_2O. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C. Lysis time can be increased up to 30 min. A longer lysis time could reduce the final yield and the quality of some viral RNA species.
3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 minutes at 95°C. (These step leads to higher sensitivity for some viral strains.)
4. Spin down unlysed material 1 min at maximum speed.
5. Transfer the **Extraction Tube** with the lysate in the **Magnet Rack** without magnet and add 400 µl **Binding Solution** and 20 µl **MAP Solution A** and mix the sample by pipetting up and down for 4- 5 times

   Note: ***Vortex the tube MAP Solution A vigorously before use!***

6. Incubate for 5 min the mixture at RT. Then insert the **Magnetic Stripe** and incubate for further 5 min at RT. Remove supernatant by aspiration or by pipetting, then remove the **Magnetic Stripe**.
7. Add 800 µl **Wash Buffer R1** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant and then remove the **Magnetic Stripe**.
8. Add 800 µl **Wash Buffer R2** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, Remove the **Magnetic Stripe**.

Repeat once this Step.

8. Insert the **Magnetic Stripe** and open the lid of the tube for 10 min to evaporate the ethanol completely.
9. Remove the **Magnetic Stripe**, then add 120 µl of the **Elution Buffer R**, mix by pipetting up and down for three times. Incubate at room temperature for 5 min.
10. Insert the **Magnetic Stripe** and incubate for further 3 min. Transfer the eluate to the 1.5 ml **Elution Tube**, centrifuge the tube for 3 min at 10.000 x g and transfer the supernatant to a new 1.5 ml **Elution Tube.** If no centrifuge is available, please put the vial again in the **Magnet Rack** insert the **Magnetic Stripe** and incubate for further 5 min at RT. Transfer the supernatant in a new 1.5 ml **Elution Tube.** It contains the viral nucleic acids ready to use.

Important Notes:

1*. The viral nucleic acids can also be eluted with a lower volume of Elution Buffer R (depends on the expected amounts of viral nucleic acids). But pay attention that minimum volume for the elution is* ***30 µl and that this volume can reduce the maximum yield****. If quite large amount of viral nucleic acids are expected, the volume of Elution Buffer can be increased up to 200 µl.*

2*. After finishing the extraction protocol, the Elution Tube contains the extracted viral RNA/ DNA. We recommend storage of the DNA at –20°C or –80°C especially for RNA.*

Protocol 4: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from plant tissue

Please read the instructions carefully and conduct the prepared procedure

1. Homogenization of 10 mg plant material with mortar and pistil under liquid nitrogen or in a mixer mill. Transfer the homogenized plant material into the **Extraction Tube.** Add 400 µl of ddH_2O. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C. Lysis time can be increased up to 30 min. A longer lysis time could reduce the final yield and the quality of some viral RNA species.
3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 minutes at 95°C.
4. Spin down unlysed material 1 min at 10.000 x g.
5. Transfer the **Extraction Tube** with the lysate in the **Magnet Rack** without magnet and add 400 µl **Binding Solution** and 20 µl **MAP Solution A** and mix the sample by pipetting up and down for 4- 5 times

Note: ***Vortex the tube MAP Solution A vigorously before use!***

6. Incubate for 5 min the mixture at RT. Then insert the **Magnetic Stripe** and incubate for further 5 min at RT. Remove supernatant by aspiration or by pipetting, then remove the **Magnetic Stripe**.
7. Add 800 µl **Wash Buffer R1** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, and then remove the **Magnetic Stripe**.
8. Add 800 µl **Wash Buffer R2** to the vial. Close the vial, and mix it by pipetting up and down. Incubate for 3 min in the **Magnet Rack** with inserted **Magnetic Stripe**. Remove supernatant, and then remove the **Magnetic Stripe**.

Repeat once this Step.

9. Insert the **Magnetic Stripe** and open the lid of the tube for 10 min to evaporate the ethanol completely.
10. Remove the **Magnetic Stripe**, then add 120 µl of the **Elution Buffer R**, mix by pipetting up and down for three times.
    Incubate at room temperature for 5 min.
11. Insert the **Magnetic Stripe** and incubate for further 3 min. Transfer the eluate to the 1.5 ml **Elution Tube**, centrifuge the tube for 3 min at 10.000 x g and transfer the supernatant to a new 1.5 ml **Elution Tube.** If no centrifuge is available, please put the vial again in the **Magnet Rack**, insert the **Magnetic Stripe** and incubate for further 5 min at RT. Transfer the supernatant in a new 1.5 ml **Elution Tube.** It contains the viral nucleic acids ready to use.

Important Notes:

1. *The viral nucleic acids can also be eluted with a lower volume of Elution Buffer R (depends on the expected amounts of viral nucleic acids). But pay attention that minimum volume for the elution is* ***30 µl and that this volume can reduce the maximum yield****. If quite large amount of viral nucleic acids are expected, the volume of Elution Buffer can be increased up to 200 µl.*

2. *After finishing the extraction protocol, the Elution Tube contains the extracted viral RNA/DNA. We recommend storage of the DNA at –20°C or –80°C especially for RNA.*

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA/RNA	insufficient lysis	increase lyses time, but prevent too long lyses tome because this also decrease yield reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer R**, be sure you pipet the Elution Buffer R with the right amount
	low amount of **MAP Solution A**	mix **MAP Solution A** thoroughly before pipeting to the sample
low concentration of extracted DNA/RNA	too much **Elution Buffer**	elute the DNA with lower volume of **Elution Buffer R**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
degraded DNA/RNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20°C)! avoid thawing and freezing of the material old material often contains degraded DNA/RNA
DNA/RNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge down at full speed for 1 min and transfer supernatant to a new tube

General notes on handling viral DNA

1) Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as, long-template PCR.

2) Storage of DNA

A working stock of DNA can be stored at 2 – 4˚C for several days. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

3) DNA yield

The amount of purified viral DNA depends on sample source, transport conditions, storage, and age of the sample.

General notes on handling viral RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240° C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37°C and then be autoclaved or heated to 100°C for 15 min to remove residual DEPC

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- **Non-disposable plasticware** should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plasticware rinsed with chloroform to inactivate RNases.
- All **buffers** must be prepared from DEPC-treated RNase-free ddH_2O.
- When **working with chemicals**, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Change gloves frequently and keep tubes closed.
- All centrifugation steps are carried out at room temperature.
- To avoid **cross contamination** cavity seams shouldn't be moisten with fluid.
- Reduce the preparation time as much as possible.
- **Use only sterile, disposable polypropylene tubes** throughout the procedure. (These tubes are generally RNase free.)
- Keep isolated RNA on ice.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.

This kit should only be used by personnel trained in *in vitro* diagnostic laboratory practice.

Storage of viral RNA

Purified viral RNA can be stored –80°C and is stable for months and years, e.g. precipated and stored in 70% ethanol.

Ordering information

Product	Package size	Catalogue No.
InviMag® Virus DNA/RNA Mini Kit	50 preparations	1440100200
InviMag® Virus DNA/RNA Mini Kit	250 preparations	1440100300

Related products

InviMag® Virus DNA/RNA Mini Kit/ KFmL	15 preparations	2441150100
InviMag® Virus DNA/RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Virus DNA/RNA Mini Kit/ KFmL	300 preparations	2441150400
InviMag® Virus DNA/RNA Mini Kit/ KF96	1 x 96 preparations	7441050100
InviMag® Virus DNA/RNA Mini Kit/ KF96	5 x 96 preparations	7441050200
InviMag® Virus DNA Kit/ KF96	1 x 96 preparations	7440300100
InviMag® Virus DNA Kit/ KF96	5 x 96 preparations	7440300200
InviMag® Virus RNA Kit/ KF96	1 x 96 preparations	7443300100
InviMag® Virus RNA Kit/ KF96	5 x 96 preparations	7443300200
RTP® DNA/RNA Virus Mini Kit	50 extractions	1040100200
RTP® DNA/RNA Virus Mini Kit	250 extractions	1040100300
Invisorb® Virus DNA HTS 96 Kit /X	4 x 96 preparations	7142310300
Invisorb® Virus DNA HTS 96 Kit /X	24 x 96 preparations	7142310400
Invisorb® Virus RNA HTS 96 Kit /X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit /X	24 x 96 preparations	7143310400

Single Components for InviMag® Virus DNA/ RNA Mini Kit

Extraction Tubes	10 Tubes	7440301500
MAP Solution A	1 ml	7440305100
Elution Buffer R	30 ml	7440304100
Wash Buffer R1 (add 80 ml ethanol)	80 ml	7440303500
Wash Buffer R2 (add 160 ml ethanol)	40 ml	7440303600

Possible suppliers for Isopropanol:

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma
2-Propanol
Order no. 59304-1L-F

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